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Advances in analysis of microplastics in drinking water treatment plants. Fluorescence techniques using iDye Pink

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ABSTRACT

Recently, the increasing amount of plastic waste has raised concerns about microplastics in aquatic environments. In this study, microplastics between 0.1 and 5 mm in samples from different points of three Drinking Water Treatment Plants (DWTP) were separated, quantified, and identified. Staining methods were used in combination with microscopic and spectroscopic techniques. On the one hand, the Rose Bengal dye was used to discriminate between natural and synthetic particles. On the other hand, Nile Red and iDye Pink reagents have been evaluated for staining microplastics, providing them with fluorescence. The nature of the particles is determined by comparison with a series of patterns by means of an epifluorescence microscope. In addition, "Micro Fourier Transform Spectrophotometer " and "Raman Spectroscopy" were used to identify the nature of the particles more accurately. Concerning the results, polyester was the most common material by fluorescence identification, and it was confirmed with Raman spectroscopy. Since most of the particles observed were microfibres, Raman proved to be a better identification technique than μ-FTIR, which could only identify large fragments. In addition, the global elimination of microplastics (MPs) resulted notable in the three DWTPs, being 81.47% for A, 88.98% for B, and 82.27% for C, thus guaranteeing a higher quality of drinking water.

1. Introduction

In the last few years, a global concern has emerged about plastic contamination in ecosystems (soil, air, marine, and fresh water). Initially, it was focused on large pieces of plastic present in the environment. However, recently, much attention has been paid to microplastic contamination. Microplastics are synthetic and have been described as emergent pollutants with a size smaller than 5 mm (Koelmans et al., 2016; Sarkar et al., 2021).

Since plastics were introduced into society in the 20th century, there have been intentional and accidental discharges into the environment, causing their accumulation and contamination in the environment, specifically in aquatic ecosystems (Barchiesi et al., 2021; Issac and Kandasubramanian, 2021). Worriedly, the presence of microplastics has been detected in drinking water, being one of the main routes through which microplastics can reach living organisms (Halfar et al., 2021).

Abbreviations: DWTP, Drinking Water Treatment Plant; MP, Microplastic; PP, Polypropylene; LDPE, Low Density Polyethylene; HDPE, High Density Polyethylene; PS, Polystyrene.

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Microparticles (all particles smaller than 5 mm) found in water can be classified, according to their shape, as microfibres, fragments, and spheres or nature since they can be made of natural, artificial, or synthetic materials. Among these three materials, only the synthetic ones (non-biodegradable polymers) are considered microplastics. These micro-pollutants are divided into primary and secondary microplastics, depending on their sources. Primary microplastics are tiny particles such as microspheres used in cosmetics, while secondary microplastics are generated by the fragmentation of larger plastics. The whole classification of microparticles is summarized in Fig. S1 of supplementary data (Ngo et al., 2019; Rosal, 2021).

Microfibres have a very small diameter (10–20 μm) compared to their length (< 5 mm). Their origin can be vegetable, animal, or other substances, such as petroleum, mostly from textiles (Barrows et al., 2018). The main source of microfibres comes from the wastewater generated at home washing machines and dyeing and finishing processes of the textile industry (De Falco et al., 2018; Güyer et al., 2016; Hernandez et al., 2017).

According to the materials that constitute microfibres, they also can be natural, synthetic, and artificial materials, as commented above. Natural microfibres are cotton, wool, linen and silk. These are more biodegradable, which can favour their elimination from wastewater. However, the release of additives contained in them, which can be harmful to the aquatic environment, is an important problem to be considered (Do et al., 2022). Synthetic microfibres are mainly of polyester and, to a lesser extent polyamide, polypropylene, and polyethylene (Mishra et al., 2020). Synthetic microfibres can be classified as secondary microplastics, which are shed from larger fabrics. Finally, artificial microfibres are made up of regenerated cellulose, such as rayon, which is regenerated through the "viscose" process. It should be noted that, until the end of the 19th century, textile fibres were only of natural origin: cotton (vegetable) and wool (animal) (Ahmed and Mondal, 2021). Nowadays, the production of textile fibres worldwide is mainly based on polyester at 52%, followed by cotton with a 24% (Textile Exchange A, 2021).

Concerning fragments, they are usually secondary microplastics that result from the fragmentation of larger objects, such as bottles, bags, containers, or tires. Fragmentation occurs by wearing these materials or once they reach the environment. The most common types of plastics from which the fragments are derived are high-density polyethylene, HDPE (plastic bags, bottle caps, plastic boxes), low-density polyethylene, LDPE (plastic bags, plastic rings for transport cans), polypropylene, PP (ropes, bottle caps), polyethylene terephthalate, PET (bottle strapping, photographic film, film) and synthetic rubber (tires) (Talvitie et al., 2017).

Although there are no sufficiently solid studies about the effects of MPs on health, numerous works indicate that they can cause problems in our body: cytotoxic effects on cells, inflammations in the intestine, imbalance of the intestinal flora, vectors for contaminants, vectors for pathogens and leaching of toxic chemical additives (Banerjee and Shelver, 2021; Schirinzi et al., 2017). Therefore, it is crucial to deepen the study of techniques for identification, separation, and quantification of microplastics in water. Thus, it is necessary to quantify the concentration of MPs and establish limit values to minimize their risks.

There is no standard method for microplastic characterization in drinking water. In fact, microplastic occurrence from drinking water samples has been studied by many authors using spectroscopic techniques, such as FTIR (Dronjak et al., 2022; Simon et al., 2018) or Raman (Kniggendorf et al., 2019; Pittroff et al., 2021). Many other studies suggest using pyrolysis coupled with gas chromatography-mass spectrometry to perform a mass quantification of MPs (Okoffo et al., 2023). These techniques require a lot of time, and expensive equipment is needed. Thus, staining methods could be techniques to be considered since they are less time-consuming and easier to implement. Rose Bengal staining has proven to be useful for MP counting from drinking water (Gbogbo et al., 2020; Lam et al., 2020), while Nile Red staining has been used for MP characterization from drinking water (Erni-Cassola et al., 2017; Maes et al., 2017). Until now, the use of iDye pink staining for MP characterization has been reported only for prepared samples of MP instead of real drinking water samples (Karakolis et al., 2019), but with prepared samples. Indeed, iDye pink could be a promising and more environmentally friendly alternative to Nile Red, for MP characterization, considering that iDye solution is prepared in water, while Nile Red solution is prepared in acetone or methanol (Alvim, 2022; Maes et al., 2017). In this work, it has been proposed using staining methods combined with microscopic and spectroscopic techniques. Specifically, for microplastic characterization, staining with iDye pink (a solution more sustainable than Nile Red solution) has been proposed as an alternative to Nile Red staining, and it has been decided to combine it with spectroscopic techniques such as FTIR and Raman, for a more precise characterization. Thus, complexity and time are notably reduced, and the microplastic analysis process is enhanced.

This work is focused on the separation, identification, and quantification of microplastics in different water samples from three different DWTP. For the first characterization of microplastics in water samples, particles are stained with Rose Bengal. After staining, a visual inspection with a stereoscopic microscope is carried out to establish a classification between natural and synthetic microparticles, and the microplastic concentration of each sample is determined. Next, using spectroscopic techniques such as Micro Fourier Transform Infrared Spectroscopy (μ-FTIR) and Raman Spectroscopy, the material of each particle is identified. Since the identification process with both μ-FTIR and Raman is slow and requires great skill in handling, a previous stage of pre-identification by fluorescence-staining methods (qualitative techniques) allows a first screening of each sample. Recent studies indicate that fluorescence staining can be performed using Nile Red reagent water (Erni-Cassola et al., 2017; Maes et al., 2017). Alternatively, a new reagent that has not been used yet to analyze water samples, iDye Pink, was tested in this study since there is evidence that it can be cheaper and more precise than Nile Red (Karakolis et al., 2019).

2. Materials and methods

2.1. Sampling sites

Samples of 5 L were collected from 5 different treatment points in each DWTP. During each sampling, first 5 L were discarded to avoid possible cross-contamination, and samples were transported in glass bottles. Raw water captured by each DWTP has a different

origin. Raw DWTP A water comes from a water reservoir, while in the case of DWTP B, it comes from an underground well. In the case of DWTP C, raw water comes from a seawater intake. Although the treatments are similar in each plant, there are slight differences between them. Fig. 2S of supplementary data summarizes the treatment processes for each plant and the collection point of the different samples.

In DWTP A (Fig. S2a) there is a pretreatment with $KMnO_4$ followed by a settling tank. Then, the water stream passes through conventional filters, and a final chlorine disinfection is done. In DWTPs B and C (Fig. S2b and c) there is also a pretreatment followed by a sand filter. The main difference in DWTPs B and C is in the filtration process preceding the reverse osmosis stage. It must be noted that in DWTP B and C, it is necessary a previous microfiltration and a remineralization by mixing with a small part of the raw water.

Table 1 S of supplementary data enumerates the different samples taken from each DWTP for their analysis.

2.2. Quality assurance and quality control

All the glass materials were carefully washed with soap, rinsed with water, and finally with ethanol to ensure the quality of the analysis minimizing contamination.

A polycarbonate filter was left on the workbench on a Petri dish for 24 h to evaluate the possible air contamination. In addition, the presence of microplastics in the deionized water used for cleaning was also evaluated by filtering 1 L through a polycarbonate filter. The filter was dried in an oven for 1 h at 60 °C. Finally, the contamination of a filter just taken out of the box was also determined. The three used filters (for aerial contamination, deionized water, and new filter) were stained with Rose Bengal. Next, microplastic counting was performed following the same procedure described in Sections 2.4 and 2.5.

2.3. Samples pretreatment and filtration

In the case of raw water samples, water chemical digestion was performed as a pretreatment to eliminate the organic matter, which could interfere with further analysis. Reaction was performed with a dilution 1:100 with H_2O_2 35 wt% (1 ml of H_2O_2 + 99 ml of sample), for 2 h and at 60 °C (Alvim, 2022; Syakti et al., 2018). The dilution 1:100 was selected to avoid the polymer degradation due to the contact with H_2O_2 (Pfohl et al., 2021; Schrank et al., 2022). Then, samples were filtered through a 10 μm pore polycarbonate filter (Whatman Nuclepore, USA).

For the rest of the samples, the digestion was considered not to be needed due to the low organic matter concentration, and samples were filtered (1 L). Each filter was introduced in a Petri dish covered with another one of a larger diameter. Then, it was placed in the oven for 1 h at 60 °C and, finally, for 1 h in the desiccator.

Samples were also characterized in terms of pH, turbidity, conductivity, and chemical oxygen demand (COD). Turbidity was measured with the turbidimeter Lange TU5200 from Hach (Spain), pH and conductivity with pHMeter GLP 21 + and EC-Meter GLP 31 + (CRISON, Spain), respectively, and COD with kits from Merck (Spain).

2.4. Staining

Each filter prepared as detailed in Section 2.3, with the microparticles of the samples, was stained with Rose Bengal (Sigma, Saint Louis, USA). Natural particles were stained, while the synthetic ones were not stained. Hence, the microplastic concentration (MP/L) was determined by counting the number of not-stained particles. On the other hand, different filters were prepared following the same procedure but, in this case, they were stained with Nile Red (Sigma, Saint Louis, USA) in DWTP A and iDye Pink (Jacquard Products, Healdsburg, USA) in DWTP B and C for fluorescence identification. In Fig. S3, it has been summarized the dye used for the staining with each sample.

The staining process consisted of pouring a few drops of a dye solution, with a dropper, on the filter, covering its entire surface. Then, the Petri dish with the filter was placed in the oven and put on the desiccator. In some cases, it was necessary to rinse after the staining. The rinsing consisted of pouring deionized water through the filter and collecting all the water in a beaker. Then, the water was filtered again through the same filter, recovering the MPs that could be washed out of the filter. Thanks to the rinsing, the contribution of the filter to the background fluorescence was minimized.

Table S2 shows the staining conditions for each tested dye and the references from previous works that suggest similar conditions.

In the case of iDye Pink, a higher concentration and temperature (100 g/L, 70 °C) were proposed by Karakolis et al. (2019). However, it was decided to use a lower concentration (1 g/L) and temperature (60ºC) since, with these experimental conditions, satisfactory results with patterns were obtained, and no differences with a temperature of 70ºC and a concentration of 100 g/L were observed.

2.5. MP counting

Microparticle counting and the determination of their morphology were carried out for the samples stained with Rose Bengal. A stereoscope (Leica MZ APO, Wetzlar, Germany) was used to observe the microparticles, with magnifications between 8X and 80X.

To improve the counting process, a transparent circular mesh divided into octants was used, according to Alvim (2022). The mesh is stuck to the Petri dish below it, making it to coincide with the filter located inside the Petri dish. Being transparent, each of the divisions and the numbers that identify them can be observed.

The counting process was divided into two parts: in the first one, a white background was used to observe the Rose Bengal-dyed

particles and other coloured particles, while in the second one, a black background was used to discriminate the white particles. All the microparticles in the 0.1–5 mm range were considered in the counting.

2.6. MP identification

This process was carried out by a previous fluorescence identification and a more precise identification by μ-FTIR or Raman in order to compare these techniques.

2.6.1. Fluorescence identification

In the case of the samples stained with Nile Red and iDye Pink, the analysis consisted of determining their nature from the fluorescence emitted.

Fluorescence was observed with the epifluorescence microscope Olympus BX50 (Olympus, Paris). It was determined by applying three excitation wavelengths, and for each one, an emission associated with a given colour was obtained. In Table S3, excitation and emission wavelengths and their corresponding colours can be observed.

Different patterns were stained for each dye: polyester, polypropylene, cotton, wool, rayon, HDPE, LDPE, polystyrene, linen, rubber, nylon, PVC. Materials were selected according to the most common textile fibres in the industry (Textile Exchange A, 2021; Ahmed and Mondal, 2021). Fluorescence identification of each stained particle then consisted of comparing the colours emitted with the patterns. Each particle was identified according to the matching pattern.

2.6.2. Raman and μ-FTIR identification

For a more precise identification of the material, Raman and μ-FTIR spectroscopy techniques have been used (μ-ATR-FTIR Bruker, Madrid, Spain), (Raman Alpha 300 R+, Ulm, Germany). The first idea was to analyze, by spectroscopy techniques, those particles that had been characterized as synthetic (possible microplastics) in the previous fluorescence identification. Thus, the number of particles to be analyzed by μ-FTIR or Raman would be reduced, and the process would be speeded up. This could be done with μ-FTIR, but in Raman the fluorescence generated much noise in the spectra, and additional unstained samples had to be prepared. μ-FTIR was used for the samples of DWTP A after the fluorescence analysis, while RAMAN was used for the samples of DWTPs B and C before their staining.

The analysis process with μ-ATR-FTIR was laborious and complex since the equipment did not include the possibility of putting the filter directly on the slide, and every particle had to be taken separately. Then, it is placed in the equipment, and the infrared spectrum is obtained. Using KnowItAll and Spectragryph software, the spectra obtained were analyzed to identify the material of the particles.

On the other hand, Raman spectroscopy allows a faster and more precise analysis. Additional samples were prepared for Raman analysis, pretreated with H_2O_2 at a ratio of 1:100 for 2 h to remove organic matter and dyes that provide fluorescence to the particles. Once the sample was prepared, the spectrum was obtained. The spectra exhibited some noise. An algorithm provided by Ghosal et al. (2018) was used to eliminate this noise, in which the multipolynomial and smoothing fit was performed using a Savitzky-Golay filter. Then, the spectrum and the main peaks were plotted to identify the material.

2.7. Analysis with FESEM-EDX

In the case of DWTP C, after filtering the first three samples, a deposit of solids was observed on the filter, making the counting process difficult, especially to observe the white microfibres and fragments. To determine the nature of these solids an analysis with FESEM-EDX Zeiss Ultra 55 (Zeiss, Oberkochen, Germany) was carried out.

The process consisted of two stages: in the first stage, an elemental analysis was carried out, in which the solids to be analyzed were selected one by one, and it was done with 446X magnification and a potential of 10 kV. The equipment provides the value of the weight percentage composition of each atom. The second stage consisted of taking images of each sample with 400X magnification and a potential of 2 kV.

2.8. Methodology overview

In the developed methodology, Rose Bengal was used to determine the concentration of microplastics (MP/L), and Nile Red was used to determine the nature of plastics based on their fluorescence. On the other hand, iDye Pink was proposed as an alternative to Nile Red. In addition, the identification of the nature of the microparticles was carried out with Raman and μ-FTIR. Fig. S3 summarizes in a block diagram the methodology applied.

3. Results and discussion

3.1. Staining of patterns (raw materials)

Tables 4 S, 5 S, and 6 S summarise the patterns stained with Rose Bengal (a), Nile Red (b) and iDye Pink (c), respectively. The inherent fluorescence of each material can be found in Table 7 S. Furthermore, in the case of the patterns stained with Rose Bengal, the RGB code has been determined. This additive colour model accurately reproduces the colour accurately by mixing the three primary light colours (red, green, and blue). Hence, it is possible to discriminate between stained and not-stained materials. For Nile Red and

iDye Pink dyes, Tables 5 S and 6 S show the fluorescence emission colour for each wavelength applied (Table S3).

The results of the Rose Bengal patterns (Table S4) agree with those of Gbogbo et al. (2020) and Lam et al. (2020) since natural and artificial particles (cotton, wool, rayon, and linen) were stained, showing a pink tone. However, synthetic or plastic particles (polyester, PP, LDPE, HDPE, PS, rubber and nylon) maintain their natural colour. According to the results, it could be considered that Rose Bengal staining is a good technique to classify particles into natural and artificial (if stained) and MPs (if not stained). Thus, by counting the non-stained microparticles with the help of a stereoscope after applying Rose Bengal, the concentration of MPs (MP/L) in the water can be determined.

In Table S7, it is presented the inherent fluorescence of each material. Most of them do not show fluorescence, but in the case of cotton, polystyrene, and nylon, these raw materials emit blue fluorescence. Comparing the fluorescence emitted after staining, the materials stained with Nile Red (Table S5) and iDye Pink (Table S6) showed similar fluorescence colours. Hence, Nile Red staining proves to be a faster and simpler technique for microplastic identification. In addition, iDye Pink staining, which has not been used yet with DWTP samples, works similarly and has some advantages compared to Nile Red. Thus, by comparing the fluorescence emitted by the stained samples taken from the DWTPs with the patterns, the nature of each sample can be qualitatively determined. Furthermore, all the patterns stained with Nile Red and iDye Pink were analyzed again 72 h later, and it was confirmed that fluorescence did not disappear. Thus, fluorescence identification does not have to be done immediately after staining.

3.2. DWTP raw water characteristics

Table S8 shows the results of the raw water analysis of each DWTP.

In DWTP A, the greatest turbidity stands out compared to the values of B and C, which can be explained since DWTP A treats surface water. On the other hand, the COD of C is the highest of the three DWTPs. In this sense, the presence of salts can interfere with the determination of COD. It is also worth noting the high the conductivity value of C since it is seawater. Turbidity values of the rest of the samples are described and commented on together with the concentration of MPs in Section 3.3.2.

3.3. MP counting

This section presents the results of MP counting in blank and water samples.

3.3.1. MP counting in blank samples

The results of the blank samples (quality assurance, see Section 2.2.) are presented in Table S9 in terms of concentration (MP/L).

The Results of Table S9 have to be considered in the characterization of all the samples. Considering that 100 ml of deionized water was used in the Rose Bengal staining process of each sample, a value of 4 MP has been considered as cross-contamination due to deionized water. On the other hand, aerial contamination was neglected since each filter is exposed to the environment for less than 5 min (immediately after staining, they were covered between two Petri dishes). Concerning the used filter, a value of 21 MP has been accounted for each new filter used (contamination related to the manipulation of the filter during the staining and counting processes). Therefore, a value of 25 MP has been subtracted from the count of each sample.

It is worth mentioning another kind of cross-contamination observed during the analysis: cork contamination fragments from the bottle plugs were observed in some samples (Fig. S10). However, since this material is natural, cork fragments were stained with Rose Bengal (Table S4), and did not affect the counting process.

3.3.2. MP counting in water samples

The results of MP counting and turbidity for all the samples of each DWTP are presented in Fig. 1.

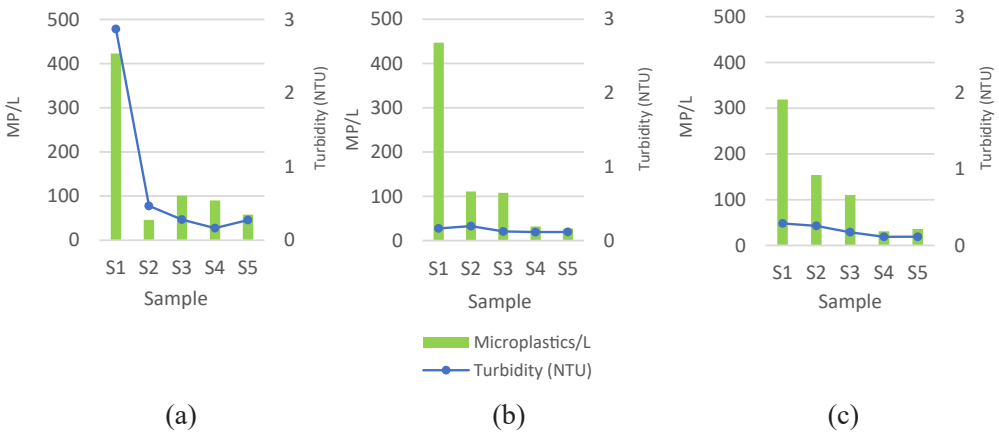

Fig. 1. Microplastic concentration and turbidity of different samples in DWTPs A (a), B (b) and C (c).

The concentration of MPs was similar for the raw water (S1) of DWTPs A and B and slightly lower in C. This may be since, in DWTP C, the presence of solids in the filter has meant that some particles could not be distinguished and have not been counted as microplastics.

The raw water sample (S1) from DWTP A (Fig. 1a), which comes from a water reservoir, had a 448 MP/L concentration. Pivokonsky et al. (2018) studied the concentration of microplastics in 2 DWTPs with water reservoir catchment water, and they obtained concentrations of 1473 MP/L and 1812 MP/L, respectively. These authors considered smaller particles (up to 1 μm) in the counting, which could contribute to the higher concentration than the value obtained in this work. In DWTP A, the stage in which the highest percentage of MPs is removed is the settling stage (between S1 and S2). In the coagulation-flocculation process, the accumulation of MPs in the flocs is favoured, enhancing their removal during settling. In the case of the "Filtration Outlet (S3)" sample, the concentration increases slightly, while the "DWTP outlet (S4)" sample showed a slight decrease of MPs (around 8%) in comparison with S3. Between the samples "DWTP Outlet (S4)" and "Storage Tank Outlet (S5)", there is a decrease in microplastics even though there is no treatment involved. Overall, between input and output, MP concentration was reduced from 448 MP/L to 83 MP/L, which represents a decrease of 81.47%. This value is in the range of the values obtained by Pivokonsky et al. (2018), with 81%m and Alvim (2022), with 88%, with similar treatment processes.

Regarding DWTP B (Fig. 1b), raw water (S1) comes from an underground well and a concentration of 472 MP/L has been obtained. Samandra et al. (2022) also observed the presence of microplastics in groundwater with a concentration of 38 MP/L. The main decrease in MP concentration occurs between the "Raw Water (S1)" sample and the "Sand Filter Outlet (S2)". In this process, the particles are retained by the sand filter, and they are removed from the water. Thus, for this DWTP and between S1 and S2, MP concentration varied from 472 MP/L to 136 MP/L, eliminating 71.19% of the MPs present in the water. For the "Microfiltration Output (S3)" sample, the concentration hardly varies (it decreases slightly from 136 MP/L to 133 MP/L). In the following stages, with membrane technology, there is a remarkable decrease in the concentration of MPs from 133 MP/L to 57 MP/L, which represents a decrease of 57.14%. Finally, between the samples "Membrane Outlet (S4)" and "Storage Tank Outlet (S5)" there is a slight decrease in microplastics (from 57 MP/L to 52 MP/L). Overall, between input and output, there is a decrease from 472 MP/L to 52 MP/, which represents a reduction of 88.98% in the MP concentration. This reduction percentage is in concordance with results published by Wang et al. (2020). The water treatment processes are similar, but instead of reverse osmosis membranes, they used granular activated carbon filters. In their work, a microplastic removal between 82% and 89% was achieved. It should be noted that, in this case, the lower limit of the size of the particles considered in the count is much lower (1 μm). Therefore, the concentration values of microplastics in this work are higher.

Related to DWTP C (Fig. 1c), the catchment water (S1) comes from a submarine seawater intake, and a concentration of 344 MP/L is obtained. Fallon and Freeman (2021) studied the concentration of MPs in seawater in Saigon Bay (Panama) and obtained a value of 107 MP/L. They analyzed particles between 10 μm and 3 mm. These authors performed a quantification using a fluorescence microscope, which may explain why the concentration is lower since the precision of this technique may be less than the staining process with Rose Bengal. Also, the Mediterranean is a less open sea than the Caribbean, which may explain its higher accumulation of MP. Between the first two samples, “Raw Water (S1)” and “Sand Filter Outlet (S2)”, the concentration of MPs decreases remarkably. During this process, the particles are retained by the sand filter, and they are eliminated from the water, going from a concentration of 344 MP/L to 179 MP/L, representing a removal percentage of 47.96% of the MPs. Subsequently, in the following sample, "Microfiltration Outlet (S3)", a slight decrease in the concentration of MPs of 18.43% is achieved (from 179 MP/L to 146 MP/L). In the following stage, the reverse osmosis membrane technology allows a great elimination in the MP concentration, varying from 146 MP/L to 56 MP/L, representing a decrease of 61.64%. Finally, between the samples "Membranes Outlet (S4)" and "Storage Tank Outlet (S5)", there is a slight increase in the concentration of microplastics (from 56 MP/L to 61 MP/L), which can be attributed to the casuistry of the sample or the experimental error. Thus, it can be considered that MP concentration does not vary. Overall, the concentration of MPs goes from 344 MP/L to 61 MP/L, representing an elimination of 82.27%. It should be noted that the percentage of microplastic removal (82.27%) is within the range reported by Wang et al. (2020): between 82% and 89%. In their case, instead of reverse osmosis membranes, they use granular activated carbon filters. Pivokonsky et al. (2022) also obtained microplastic removal percentages within the same order of magnitude, around 80%. Therefore, the results obtained in this work agree with the values obtained by other authors. Gbogbo et al. (2020) obtained a slightly higher removal percentage of 97% after reverse osmosis membranes.

It should be noted that the membrane processes of DWTPs B and C have produced water with the lowest values of MP concentration. In addition, for the 3 DWTPs, most of the observed particles (natural and synthetic) present a fibre morphology (between 80% and 90%), while some fragments have been found to a lesser extent (10–20%), and no microspheres have been found. On the other hand, most of both microfibres and fragments (70–80%) have been classified as synthetic materials (microplastics). In addition, most of the observed particles were white, unstained microfibres. According to Fig. S11, most of the observed microplastics were fibres (84–94% for DWTP A, 85–92% for DWTP B and 85–89% for DWTP C), while fragments have been found to a lesser extent (6–16% for DWTP A, 8–15% for DWTP B and 11–15% for DWTP C).

The relationship between the concentration of microplastics and turbidity in each DWTP has also been studied. In the case of DWTP A (Fig. 1a), a decrease in turbidity is observed with a decreasing concentration of microplastics, especially between the first two samples. For the rest of the samples, turbidity present very small values. It should be noted that, for all the samples, it was observed that the higher the concentration of MPs, the greater the turbidity. Nevertheless, in DWTP B (Fig. 1b), the turbidity values obtained were very low in all samples. All the values ranged between 0.165 (S1) and 0.117 NTU (S5), meaning that turbidity was practically constant. This result implies that there is no relation between turbidity and concentration of MPs since a low turbidity of 0.165 NTU and a high MP concentration of 472 were measured in S1, showing a very different behaviour from that commented for S1 in DWTP A. The explanation for this is that the contribution of natural organic matter in surface water contributes to the water turbidity to a greater

extent than MPs. The COD of the reservoir water was considerably higher than the COD of the well water as described in Table 5. Finally, in DWTP C, low turbidity values have been obtained in all samples, ranging between 0.289 and 0.114 NTU. The behaviour is very similar to those observed in DWTP B.

3.4. Fluorescence analysis

As explained in the materials and methods section, fluorescence was used to study the characterization of MPs with this technique as an alternative to μ-FTIR and Raman spectroscopy. Examples of fluorescence emitted by microparticles stained with Nile Red (DWTP A) and iDye Pink (DWTP B) are shown in Fig. S5a and S5b, respectively.

The materials of the microparticles found by fluorescence analysis for samples S1 and S5 in DWTP A are presented in Fig. 2 (results of samples S2, S3, and S4 are shown in Fig. 6S of supplementary data). These results have been obtained by comparing with the fluorescence of the pristine materials (Section 3.1). In this case, samples were stained with Nile Red.

The first result highlighted from Figs. 2 and S6 is that the diversity of materials decreases in the sample taken in the last sampling point of the DWTP A. Only 6 materials were identified in S5, while 11 materials were identified in S1. In "Raw water (S1)" sample, the predominant material is nylon, in a proportion of 20%. In the second sample, “Settling outlet (S2)”, the most common material was wool, at 33%. In the third sample, "Filtration Outlet (S3)", cotton, nylon, and wool stand out at 22%. In "DWTP Outlet (S4)",polypropylene stands out at 37%, while in the last sample, "Storage Tank Outlet (S5)", the main material was polyester, at 33%. Therefore, there is variability from one sample to another as it could be expected since microparticles from different materials present different characteristics. Remarkably, there is no PVC and LDPE microparticles in S5, which is because these microparticles are mainly fragments and they are more easily separated than microfibres. Thus, the percentage of polyester microfibres increases from S1 to S5.

In the case of DWTPs B and C samples were stained with iDye Pink. The results of fluorescence identification of DWTPs B, and C are presented in Figs. 3 and 4, respectively (the results of samples S1 and S5 are shown, while the results of the resting samples are presented in Figures 7 S and 8 S of supplementary data).

In Figs. 3 and S7, the predominant material in most of the samples is polyester (between 12% and 48%). Compared to DWTP A, a smaller variety of materials has been identified in S1, which could be expected since DWTP B treats well water. However, for the rest of the materials, the percentages are quite different. As in DWTP A, no microparticles of LDPE and HDPE have been found in the last sampling point (S5) since fragments are easily removed compared to microfibres. Again, the percentage of polyester microfibres increases from S1 to S5.

Figs. 4 and S8 show that the predominant material in all samples is also polyester (between 28% and 55%). It reinforces the conclusion that polyester microfibres are difficult to eliminate in DWTPs. In this case, the same variety of materials appears in S1 and S5. As in the other DWTPs, PVC and HDPE appear in S1 but not in S5. Surprisingly, microparticles of LDPE are present in S5 in the same

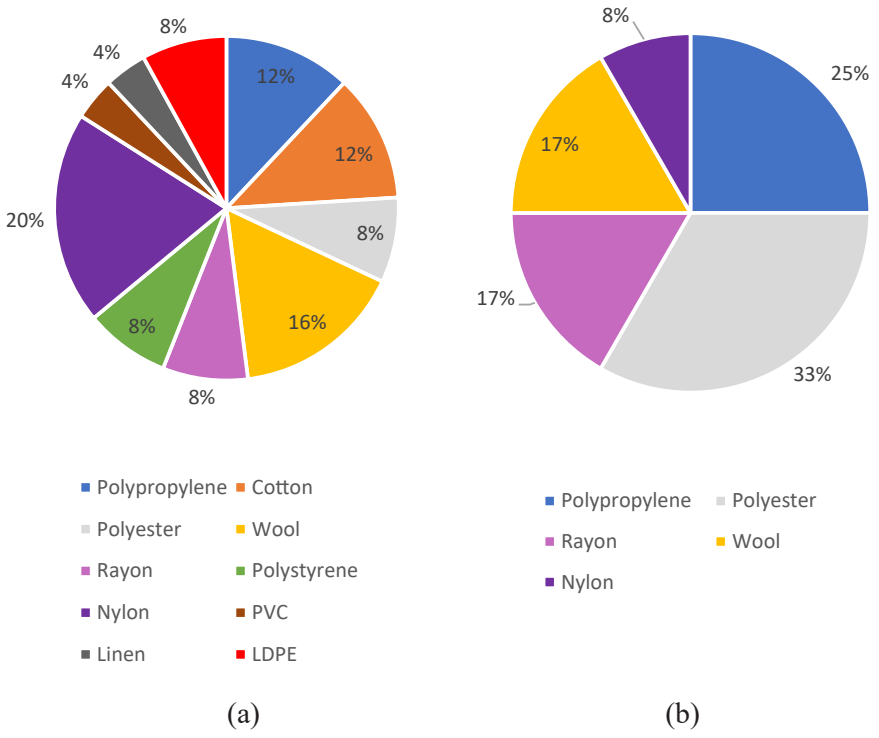

Fig. 2. Fluorescence identification of samples S1 (a) and S5 (b) from DWTP A.

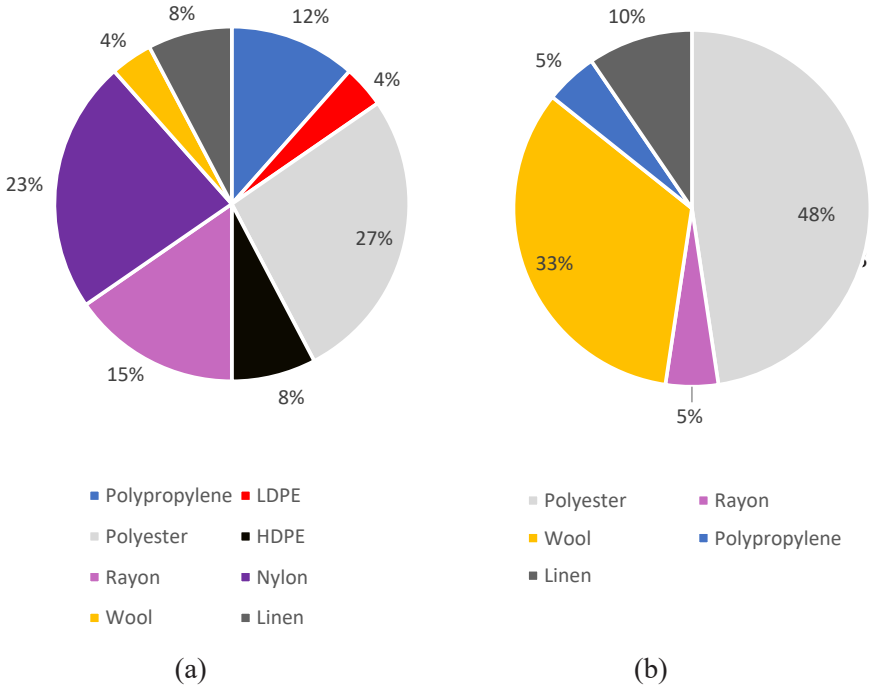

Fig. 3. Fluorescence identification of samples S1 (a) and S5 (b) from DWTP B.

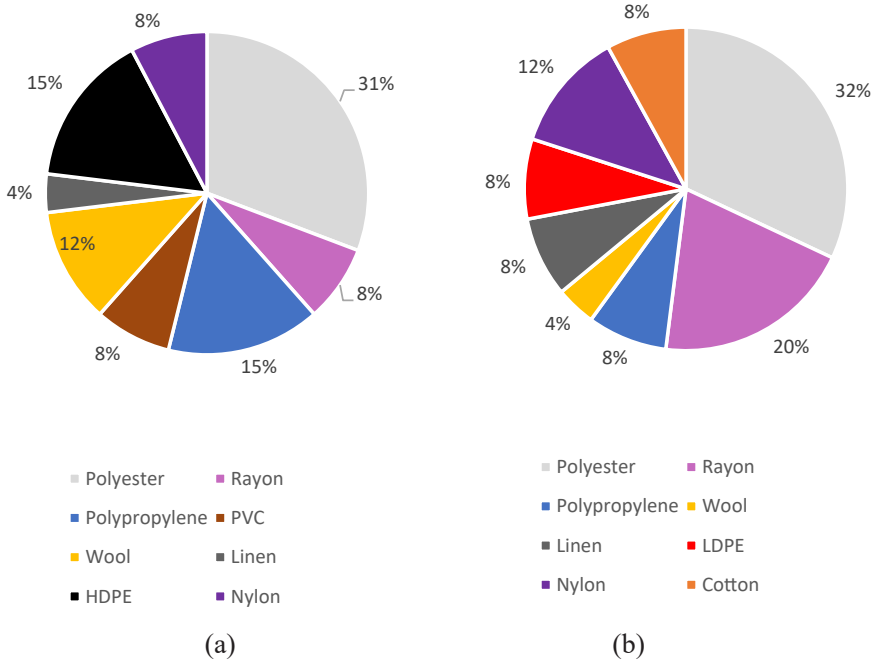

Fig. 4. Fluorescence identification of samples S1 (a) and S5 (b) from DWTP C.

percentage as linen and polypropylene.

It should be noted that in some cases (a mean value of 12% in DWTP A, 18% in DWTP B, and 8% in DWTP C), the microparticles did not show fluorescence. Thus, they could not be related to any of the patterns. The degradation process of the microparticles in the water may decrease their fluorescence. In addition, it has been observed (Tables 5 S and 6 S) that iDye Pink offers less ambiguity than Nile Red, proving that iDye Pink allows a better identification of materials. In the case of Nile Red, some ambiguities due to similar fluorescence patterns were observed between polyester-rayon, linen-wool, PP-HDPE-PVC. With iDye Pink, the differences in the

fluorescence between those patterns facilitated the distinction of these materials, and there was no ambiguity. For example, between polyester and rayon the blue emission was notably different. The same difference in blue emission was observed with linen and wool. Moreover, PP showed no fluorescence, which enhanced the distinction between HDPE and PVC. In addition, iDye Pink is cheaper (Karakolis et al., 2019), and has lower toxicity than Nile Red, since Nile Red is dissolved in organic solvents, whereas iDye Pink is dissolved in an aqueous solution.

3.5. Spectroscopic analysis

In the case of DWTP A, the analysis of the polymeric chain was carried out using μ-FTIR. For each microparticle, a spectrum was obtained to identify the material. Due to the complexity of the process of picking up the microparticles with the tweezers, placing them on the μ-FTIR slide, and applying the germanium tip of the equipment to the sample, it has not been possible to analyze all the microparticles that were selected in the previous section. In addition, the spectrum obtained did not present defined peaks in some cases. Thus, it was easier to analyze with μ-FTIR fragments, and it was more complicated in the case of microfibres.

In all the samples, most of the microparticles were microfibres and, as it has been observed, the identification of the microfibres using μ-FTIR was very complex. For this reason, in the following DWTPs the analysis of the polymer chain was performed using the Raman spectroscopy technique. According to Käppler et al. (2016), this technique allows the analysis of smaller microparticles.

Fig. 5 summarizes the classification of the microparticles analyzed from "Storage Tank Outlet" samples in DWTPs B and C with Raman and fluorescence.

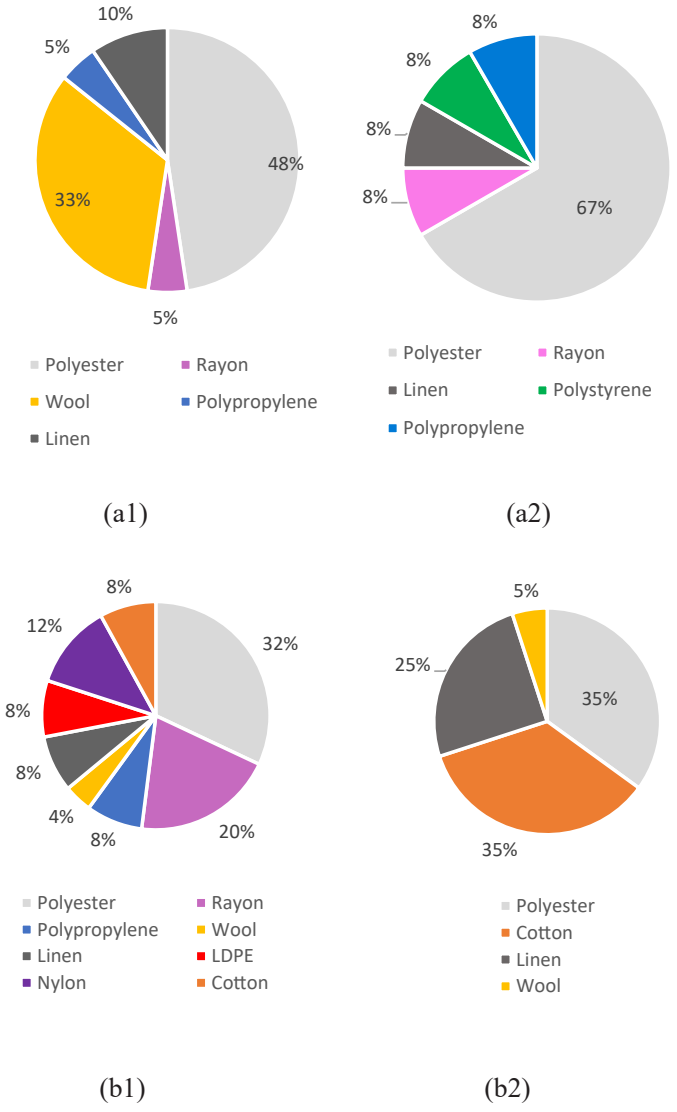

Fig. 5. Results of fluorescence and Raman identification for DWTP B (a1), (a2) and DWTP C (b1), (b2), respectively.

Fig. 5 shows that polyester was the most often identified material by Raman in DWTP B and DWTP C (62% in DWTP B and 35% in DWTP C), and matches the results obtained with fluorescence. Moreover, in DWTP B (Figures 5a1 and 5a2), linen, rayon, and PP were identified in similar proportions with both Raman and fluorescence. In DWTP C (Figures 5b1 and 5b2), wool was also observed in the same proportion with both techniques.

Regarding Raman results (Figures 5a2 and 5b2), rayon and cotton were also found in both cases, with a percentage between 8% and 35%. Considering that both materials are based on cellulose, they present a remarkably similar spectrum when using Raman spectroscopy. In addition, linen was also found in both DWTPs. While in DWTP B, polystyrene and polypropylene were also identified, and in DWTP C, only one wool fibre was identified.

Raman has been satisfactory as an alternative technique to μ-FTIR, since clear spectra have been obtained from microfibres. The digestion treatment proposed in this work has notably reduced the noise in spectra. In addition, a mathematical algorithm has been applied to minimize the noise of the spectra, enhancing the identification of the peaks.

3.6. FESEM-EDX analysis (DWTP C)

Since the feed to DWTP C was seawater, implying a high concentration of salts, inorganic microparticles were also observed. Thus, it was necessary to study it using FESEM-EDX. This technique allows the analysis of the solids deposited on the filter's surface.

Fig. 6 shows the images taken with FESEM-EDX. It is possible to observe two different shapes of solids: (a) crystals, (b) plaques.

According to Fig. 6, analysis using FESEM-EDX has revealed that the solids had a crystal morphology for the S2 sample, while for the S3 sample, they presented a plaque morphology. On the other hand, for the S1 sample, the two morphologies can be observed, both crystals and plaques. An elemental analysis has revealed the nature of each one (Table S11). The observed solids could be calcium oxide, in the case of crystals, and silicon oxide, in the case of plaques. Other elements also appear to a lesser extent, such as magnesium.

4. Conclusions

In this work, it has been developed a methodology for the separation and identification of microplastics in samples taken from five different points of the treatment of three different DWTPs. Since there are no standardized protocols and it is highly likely that a regulation will be created in the future, a deep discussion about possible quantification methods, identification and analysis of microplastics is remarkably interesting for the near future.

Staining with Rose Bengal has proved to be a simple method to determine the concentration of microplastics, since the Rose Bengal only tints natural microparticles. The results showed that, in DWTP A, MPs are mainly removed is the settling stage, whereas in DWTP B and C, after the sand filtration process. It should be noted that the global removal of MPs is notable in the three DWTPs, being 81.47% for A, 88.98% for B, and 82.27% for C.

Concerning fluorescence emission analysis, results have proved to be more qualitative than spectroscopic techniques. Within the tested dyes, iDye Pink has proved to offer less ambiguity in terms of material identification than Nile Red, being also cheaper and more environmentally friendly, which is remarkably interesting.

In DWTP A, after staining with Nile Red, it has been observed that there was no predominant material. In the case of DWTPs B and C, staining was carried out with iDye Pink, and the most common material found was polyester. In general, fragments were more easily separated than microfibres, and the percentage of them decreased from S1 to S5 samples.

The approach of carrying out a first screening using fluorescence, to later confirm the nature of the microparticle's material by means of spectroscopic techniques is possible, but μ-FTIR technique resulted more complex for the analysis of microfibres, whereas it was more suitable for fragments. In the case of Raman, post-fluorescence analysis was not possible due to the noise contributed by fluorescence, and additional samples must be prepared. However, Raman allowed microfibres to be analyzed with less complexity and without using staining. In DWTPs B and C, Raman analysis was performed, and polyester was the most present material that matched the fluorescence results.

Finally, in seawater samples (DWTP C), deposits of calcium oxide and silicon oxide solids occurred in the filter, and the quantification of microplastics was more complicated, since the solids deposited on the filter hindered the microparticle observation.

Staining methods have proved useful for MP counting (Rose Bengal) and identification (Nile Red) from drinking water, being faster and more simple than spectroscopic techniques. Moreover, iDye pink staining has proved to be a good alternative to Nile Red in terms of material identification, being also cheaper and more environmentally friendly than Nile Red. Therefore, further study would be interesting to have a more automated methodology for the MP identification process using iDye Pink.

CRediT authorship contribution statement

Pablo Alonso-Vázquez: Investigation, Methodology, Data curation, Writing – original draft. **María-José Luján-Facundo:** Supervision, Conceptualization, Methodology. **Beatriz Cuartas-Uribe:** Supervision, Conceptualization, Methodology, Writing – review & editing. **Amparo Bes-Piá:** Supervision, Conceptualization, Writing – original draft, Writing – review & editing. **José-Luis Alonso-Molina:** Supervision, Conceptualization, Methodology. **José-Antonio Mendoza-Roca:** Supervision, Conceptualization, Methodology, Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

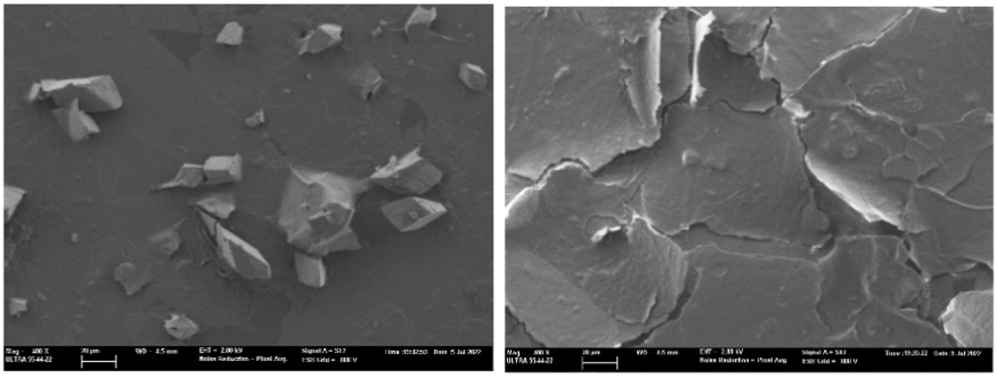

Fig. 6. Images taken of S1, S2 and S3 with FESEM-EDX (400X) of the solids deposited on the filters (a) crystals, (b) plaques.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eti.2023.103430.

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